

Biology of *Bemisia*

Session I

Biology of *Bemisia*

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Although not entirely representative of aleyrodids, the life history of *Bemisia tabaci* deserves our attention from both the biological and commercial perspective. Whiteflies are synovigenic, that is, they continue to produce eggs following adult eclosion. Questions remain as to realized fecundity; some say as few as 75 viable eggs, others report hundreds. Sex ratios are at parity in the egg stage, but may change with life stage and environmental circumstances. Eggs are attached to leaves by pedicles, through which plant material is imbibed. First instar nymphs are mobile. Later nymphal instars have been observed moving slightly during the transitional stage between stadia. The fourth nymphal instar is unusual since it is during this stage that these hemimetabolous insects (*sensu* Chapman 1998) change from a nymphal state into adulthood. This is completed under a cuticular test and wrongly called the 'pupal' stage. *Bemisia* produce a great deal of wax, mostly alkanes. In the adult stage these are distributed as particles over the body. The word root aleyro - means flour or meal. As with other instars, adults have a dorsal vasiform orifice, which 'flips' honeydew, excreta, away. *Bemisia* honeydew is composed to a large extent of trehalulose, a disaccharide, which is thought to aid in osmoregulation. Adults have remarkable abilities to disperse (at least 7 km in a few hours), which adds to their pest status and affects the ability of certain natural enemies to impact populations. In terms of flight mechanics, *Bemisia* relies on the 'clap and fling' mechanism. Wingbeat frequencies are not fixed and vary, at least, with gender and temperature. Great progress has, and continues, to be made in the area of *Bemisia* biology, the key to whitefly management.

Markers for the identification of biotypes of *Bemisia tabaci*: the Mediterranean basin as a case study

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Understanding the high degree of genetic variation within the *Bemisia tabaci* species complex is essential for interpreting the many aspects of this whitefly that contribute to its important pest status. In the early 1990's, the economic impact of the 'B' biotype, prompted the development of diagnostic techniques for its rapid identification. The first diagnostic method was the use of PAGE to produce non-specific esterase banding patterns. Used to analyse many populations collected worldwide, it enabled the description of 19 biotypes that were characterised by the letters 'A' to 'N'. The first DNA marker to be used to identify biotypes was RAPD-PCR. It corroborated the esterase studies yet simplified the experimental process for biotype identification. The later use of AFLPs, produced similar results to the RAPDs, but allowed the use of larger sample sizes in the analysis of populations. The application of another type of genetic markers, the sequence of the mitochondrial gene of the cytochrome oxidase I (COI) and the internal transcribed spacer of the rDNA region, provided an entirely new perspective of *B. tabaci* phylogeny. Using these markers, six well supported phylogenetic clades, with a clear geographic distribution at a continental level, were identified within the *B. tabaci* species complex. Finally, microsatellite markers have recently been investigated, allowing new insights into the genetic structure of populations. All the above markers have been used to investigate the degree of genetic variation of *B. tabaci* around the Mediterranean basin. This has led to the identification of four different biotypes belonging to three different clades.

Endosymbionts associated with *Bemisia tabaci* (Genn.) – a survey of global populations

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Whiteflies (suborder Sternorrhyncha, family Aleyrodidae) are known to harbor prokaryotic symbionts, some of which are vital and provide specific nutritional needs, while others are transient or nonessential, that can either be beneficial or deleterious in the long term. However, the extent to which diverse bacterial symbionts are associated with populations of the same species of whitefly that colonize herbaceous plants in diverse habitats, and their particular influence on the evolution of the whitefly host, are not well studied. Here, the composition and diversity of prokaryotic symbionts associated with biotypes and/or haplotypes of the whitefly *Bemisia tabaci* Gennadius were examined for collections from representative host plants and different geographical locations worldwide. The eubacterial 16S ribosomal DNA (rDNA) and Wolbachia-specific 16S rDNA genes for endosymbionts were obtained by polymerase chain reaction (PCR) amplification. Amplification and comparison of 16S rDNA sequences revealed that a primary-like symbiont was associated with all whitefly collections examined. However, the endosymbiont 16S rDNA phylogeny was not strictly concordant with the phylogeographically informative cytochrome oxidase I tree for the respective whitefly hosts. Secondary symbiont sequences for 13 of 20 whitefly populations clustered with *Arsenophonus* spp. and aphid T-type bacteria, which both belong to the Enterobacteriaceae. PCR and sequencing of Wolbachia-specific 16S rDNA revealed that at least 33% of *B. tabaci* populations harbored Wolbachia.

The whitefly nymphal-adult molt--identification of the molting hormone

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A staging system based on increasing body depth and the development of the adult eye has been used to identify physiologically synchronous populations of 4th instar and pharate adult silverleaf (SLWF) and greenhouse (GHWF) whiteflies. In last instars of these whiteflies, ecdysteroid titers peak just prior to and at the beginning of adult formation, at a time when body depth has achieved its maximum dimension. Although peak titers in the GHWF and the SLWF, respectively, are only 120 and 400 fg/ug protein, we have been able to use reverse phase and normal phase HPLC coupled with an exceptionally sensitive enzyme immunoassay to identify ecdysteroids that are present in the nymphal-adult premolt ecdysteroid peak. In both the GHWF and the SLWF, only ecdysone and 20-hydroxyecdysone were detected in significant quantities. The predominant ecdysteroid was 20-hydroxyecdysone, an ecdysteroid that has been reported to initiate the molt in other orders of insects. No makisterone A (reported to be the molting hormone in some hemipterans and hymenopterans) was detected.

Whitefly morphology: an ultrastructural study

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Electron Microscopy has been used to study silverleaf whitefly adult and nymph mouth parts, the characteristics of stylet penetration into host leaf tissues, and the role of leaf surface structures in feeding behavior. Most penetration sites on host leaves resulted in stylets passing directly through epidermal cells rather than through the common radial walls between adjacent epidermal cells. After passing through the epidermis, the stylets traverse the intercellular spaces between mesophyll parenchyma cells en route to the phloem tissue. The mechanical force for stylet penetration results from a change in position of the whitefly head in relationship to the labium. Stylets of both silverleaf whitefly adults and nymphs were found to be 200 micrometers in length, which is long enough to reach the phloem tissue from any position on the abaxial surface of cotton leaves. Although, surface cues may be used in whitefly feeding, they are not necessitated by stylet length. Scanning and transmission electron microscopy was used to determine the morphology of wax particles of the silverleaf (*Bemisia argentifolii*) and the giant whitefly (*Aluerodicus dugesii*) and to describe the morphology and ultrastructure of wax glands. The ultrastructural characteristics of the whitefly egg pedicel including how it is attached to the epidermal layer of the host and the functions of its fibrous ultrastructure in water uptake and translocation has been determined

Molecular phylogeography and evolution: a global assessment of *Bemisia tabaci*

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Recently, several studies have sought to use molecular markers to explore the relationships between populations of *Bemisia tabaci* from several continents. Those markers have been both nuclear (ribosomal internal transcribed spacer I or ITS1, RAPDs) and mitochondrial (16S ribosomal subunit, cytochrome oxidase I or COI) in nature. No investigation, however, has utilized both nuclear and mitochondrial data from the same populations on a large scale. We chose representative populations from six continents and obtained COI, ITS, and microsatellite sequences for analysis. Comparisons were based on each marker separately, ITS1 and COI in combination, and utilized distance and parsimony analyses, and network spanning. Although all data sets identify very well defined groups, we were unable to derive or reconstruct hierarchical relationships on a global scale. That is, phylogenetic affinities were not tree-like, nor were ancestral clades clearly identifiable. In the broad sense microsatellites supported the major clades with variable allele frequencies suggesting local founder and bottlenecking events. The relationships between geographic populations are complex and undoubtedly reflect economic migrations and introductions as well as varying local selection forces. Herein we discuss the effects of phylogenetic noise on a global resolution, and examine the status of *B. tabaci* under several species concepts.

B type vs AUS type - How many Bs do you need before establishment occurs?

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There are two biotypes of *Bemisia tabaci* in Australia. The exotic B biotype and an Australasian-Oceania biotype. In areas where the latter is abundant, the B biotype has failed to become numerous whereas in areas where it is uncommon, the B biotype has become a considerable problem. This research has shown that the interaction between host plant and the relative numbers of B type to the Australasian-Oceania biotype has a considerable effect upon the capacity for the B biotype to establish.

Molecular variability of cassava *Bemisia tabaci* genotypes in east and central Africa

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Unprecedented upsurges in *Bemisia tabaci* vector populations have been associated with the cassava mosaic disease (CMD) pandemic affected zone in East and Central Africa. Cassava plants colonised by *B. tabaci* have suffered major yield losses ranging from 20% to 100% depending on the susceptibility of the variety to whitefly-transmitted cassava mosaic geminiviruses (CMGs). Outbreaks of the whitefly vector, particularly in areas where it was previously considered unimportant, have been linked to the appearance of a new genotype of *B. tabaci*. The upsurgence of *B. tabaci* continues to be reported in areas newly affected by the CMD pandemic, and a definitive answer is needed regarding the existence (or otherwise) of a 'pandemic biotype'. The aim of our study is to investigate the genetic diversity of *B. tabaci* populations in East and Central African cassava plantings using molecular markers to detect and 'track' 'pandemic haplotypes'. The application of genetic markers that permits rapid identification of distinct haplotypes (and phenotypes) that influence the transmission and spread of CMG's will aid in forecasting patterns of present and future spread of the CMD pandemic, and lead to more sustainable control measures to combat disease spread.

RAPD-PCR characterization of *Bemisia tabaci* (Gennadius) populations in the Canary Islands

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The technique of random amplified polymorphic DNA (RAPD-PCR) is being used for biotyping *Bemisia tabaci* (Gennadius, 1889) populations from the major horticultural production areas in the Canary Islands. Previous reports have shown the presence of biotype B and Q of *B. tabaci* in the archipelago (samples taken in the period 1995-1998), therefore present situation remained to be determined. To establish the presence and distribution of *B. tabaci* biotypes in different geographic areas, surveys were made during the period 2000-2002. *Bemisia tabaci* populations have been seasonally sampled from a number of plots, both outdoors and protected tomato and cucurbit crops. With the objective of studying the role that the biotype status of *B. tabaci* populations may play in the epidemiology of viruses it transmits, the study combines the assessment of *B. tabaci* transmitted-virus incidence and the presence of the different biotypes. The present survey indicates important differences within the three major horticultural production areas in Tenerife Island. While biotype B predominates in *B. tabaci* populations from the North coast horticultural areas, biotype Q is more extended and represents the 95% from populations collected in the south and south-west coast horticultural areas, where incidence of TYLCD is higher.

Molecular characterisation of indigenous and B-biotype *Bemisia tabaci* across India

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Bemisia tabaci were collected throughout India from eggplant, tomato, tobacco, cotton and pumpkin, as well as from five weed species. Total DNAs were extracted from individual whiteflies and subjected to RAPD-PCR using OpB11 and OpB20 primers to test for the presence of B-biotype like banding patterns. Partial mitochondrial cytochrome oxidase I gene sequences (~860 bp) were then amplified from the DNAs of individual whiteflies representative of the different RAPD-PCR banding patterns obtained. Partial COI gene sequence analyses of these PCR products supported RAPD-PCR data indicating the presence of the B-biotype at Nagamangala (South India) and at Gujarat Agricultural University and at Daboi, Gujarat State (North India). Nagamangala *B. tabaci*, when fed on the zucchini plants vars. Fordhook and Ambassador, produced distinct silverleaf symptoms and the North Indian B-biotype whiteflies are currently being tested for this property. Partial COI gene sequences of Nagamangala and Gujarat clustered with those of phytotoxicity-inducing B-biotype reported previously to have been introduced to Kolar and to belong to the Old World group 3. The Gujarat and Nagamangala partial COI gene sequences have 99% and 97.5% nucleotide identity, respectively, with B-biotype sequences from Argentina (AF340216). Partial COI gene sequences of the indigenous *B. tabaci* clustered into five sub-groups within the Old World group 2. Only one of these five clusters showed host specificity.

***Bemisia tabaci* honeydew and cotton lint contamination**

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Sweetpotato whitefly, *Bemisia tabaci* (Gennadius), honeydew contaminated cotton, *Gossypium hirsutum* L., is difficult and sometimes impossible to process at the textile mill. Honeydew deposits remain localized on lint. When they adhere to machinery surfaces during harvesting, ginning, or in the textile mill, lint processing is slowed, extra machinery cleaning is necessitated, and work stoppages may occur. The major sugars found in *B. tabaci* honeydew when feeding on cotton were found to be sucrose, glucose, fructose, and trehalulose as well as the oligosaccharides stachyose, raffinose and melezitose. Carbohydrate compositions of phloem feeding insects are influenced by both insect species and host plants being fed upon. Trehalulose, turanose, palatinose and sucrose applied individually in aqueous solution sprays to cotton have been described as very sticky and melezitose, raffinose, glucose, and fructose as relatively nonsticky. The thermodetector for determining cotton lint stickiness was developed by the Institute for Research on Cotton, Montpellier, France. It has been selected by the International Committee on Cotton Testing Methods of the International Textile Manufacturer's Federation as the international standard. Increasing levels of cotton lint stickiness, as determined using a thermodetector, and increasing amounts of the *Bemisia*-produced trehalulose and melezitose are significantly correlated to increasing *B. tabaci* nymph and adult populations. Threshold levels of lint stickiness occur when leaf-turn counts of *B. tabaci* are about 8.9 or higher and nymphs per leaf-disk about 3 or more per square centimeter of cotton leaf disk. These values fall below the action threshold leaf turn count of 5.0 and nymph counts of 0.5 to 1.0 per leaf disk for effective insect growth regulator application to protect cotton yields. The results suggest that sweetpotato whitefly control action initiated to protect cotton yield also protects cotton from honeydew contamination.

New correlations between electrical penetration graph (EPG) waveforms and *Bemisia* adult and nymphal feeding behavior

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Feeding behavior of *Bemisia argentifolii* nymphs was studied using an DC electrical penetration graph (EPG). Three waveforms were identified in the EPG recordings. Pathway phase (when the stylets advance through plant tissue in an intercellular pathway) was correlated with waveform C. Phloem phase (when the stylet tips are inserted into a sieve element) consisted of two waveforms: high frequency (HF), and low frequency (LF). Using honeydew clocks, the HF waveform was clearly identified as the phloem sap ingestion waveform. No honeydew was produced (consequently little or no sap ingestion occurred) during the LF waveform. The LF waveform was always the first waveform produced during phloem phase and it alternated regularly with the HF waveform. Our working hypothesis is that the LF waveform is produced when the whitefly salivates into the sieve element. Feeding behavior of adult *B. argentifolii* whiteflies was studied using an AC electrical penetration graph (EPG). Two characteristics of the pathway phase (sawtooth waveform) were shown to be correlated with the rate and length of stylet advancement during stylet penetration into plant tissue. The rate of stylet penetration was significantly and positively correlated with frequency of sawtooth waveform voltage peaks ($r^2 = 0.33$) and the length of stylet penetration was significantly and positively correlated (second order polynomial) with the relative difference in voltage level between the beginning and end of the sawtooth waveform ($r^2 = 0.43$). Stylet advancement did not appear to occur during the few low-flat waveforms (unknown behavioral correlation) and high-flat waveforms (phloem phase) that were observed. Voltage drops occur sporadically during sawtooth waveforms, and these were associated with partial stylet withdrawal with an accuracy of 99%.

Australian whiteflies: a guide to the identification of exotic and native species

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This study aims to provide an interactive guide for the identification of exotic and native whiteflies (Aleyrodidae) found in Australia. It incorporates existing taxonomic studies with colour images of lifestages and slide prepared material. Numerous pest species are now present in Australia including *Bemisia*, *Trialeurodes* and *Aleyrodes*. Rapid and accurate identification of these species will aid those persons making control and/or quarantine decisions. Australia also contains a wide diversity of native Aleyrodids occupying habitats ranging from semi-arid shrublands through to rainforest. Recent efforts have identified numerous new taxa and undoubtedly many more species await discovery. Photographic images of many of these species are presented for the first time.

Wolbachia-induced cytoplasmic incompatibility in *Bemisia tabaci*?

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Wolbachia are a group of intracellular maternally inherited bacteria that are able to invade and maintain themselves in numerous invertebrate host species by manipulating their reproduction. Wolbachia-associated reproductive alterations include: the induction of parthenogenesis; feminization of infected genetic males to reproductively competent females; male-killing; and most commonly inducing cytoplasmic incompatibility (CI), a form of embryonic lethality in crosses between infected males and females of different Wolbachia infection status. In the present study, we used a PCR assay to test for the presence of Wolbachia in three different biotypes of *Bemisia tabaci* in Spain. Wolbachia was present in S biotype and absent from B and Q biotypes. Single pair crosses between individuals of the three biotypes indicated that mating incompatibilities observed might be due, at least partially, to the presence of Wolbachia. Therefore, the bacterium Wolbachia should now also be taken into account when describing *B. tabaci* and its different but distinguishable biotypes. This is important because the infection status might have a role in the evolution of a species complex. We also studied the distribution of Wolbachia in the soma and the germ-line of infected *B. tabaci* using an antibody against the major Wolbachia surface protein. Our data suggest that Wolbachia infections might have a role in the evolution of the *B. tabaci* biotypes or species complex. The presence of Wolbachia in both somatic and gonadal tissues indicates that these bacteria could be used for the control of whiteflies and whitefly-transmitted viruses.

Cysteine proteases in the whitefly, *Bemisia tabaci* (B-type)

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Previously, we identified proteolytic activity in whitefly whole-body extracts and demonstrated optimal activity at pH 5.5. Trypsin and chymotrypsin inhibitors were tested and found to be ineffective toward this proteolytic activity. Enzymatic activity was found in whole-body whitefly extracts using native and denaturing, reducing gelatin/polyacrylamide gel electrophoresis assays. Based on the acidic nature and the molecular weight of 40 kDa, we hypothesize that the activity is a cysteine protease. Pseudo-two dimensional gel electrophoresis confirmed that the activated protease is a single polypeptide with a molecular weight similar to that of the insect cysteine protease, cathepsin B. Using localization of enzyme activity in insect tissues, future studies will focus on determining if the protease has digestive and/or homeostatic functions.

Preliminary characterization of *Bemisia tabaci* and a geminivirus from tomato in Mali

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A survey of the presence of *Bemisia tabaci* and tomato geminiviruses in Mali was made in 2001 and 2002. Samples were taken in Katibougou (east of Bamako) and Yanfolilla (south of Bamako). The species has been found on cotton, okra, pepper, aubergine, djakatou (local aubergine) and tomato. Field surveys suggests that during the wet season (from the end of June to September) *B. tabaci* develops mainly on cotton, which is cultivated in southern and southwestern Mali, while during the dry season (from October to June) it develops on vegetables grown along rivers and ponds. The study of the population dynamics throughout the year by using yellow sticky traps is in progress. A population collected in Katibougou on cotton (K) was reared and several biological parameters characterized. The K population developed well in all the mentioned hosts as well as on *Datura stramonium*, cucumber, zucchini, French bean and failed to develop on two Compositae species (lettuce and daisy). The same population was unable to induce the squash silverleaf reaction to zucchini plants in eight repeated experiments. Developmental time from egg to adult at 25 °C constant temperature, 70 % RH and 16:8 L:D fotoperiod was of 21.66 ± 2.88 days on cotton, with an identical value on cucumber. Prolificity of newly emerged females in the same experimental conditions were of 7.48 eggs/day on cotton and 9.36 eggs/day on cucumber. In controlled transmission trials from tomato to tomato, with 3 insects per plant, K population transmitted TYLCV to 11 plants out of 20 and TYLCSV to 20 out of 50. In order to determine the biotype of the whiteflies, a 503 bp fragment of DNA of the mitochondrial COI gene was sequenced in six individuals. The BLAST alignment indicated that these insects belong to the Mediterranean-African clade, with the highest match (98 %) with GenBank sequences of samples from Morocco and Spain. Using generic begomovirus primers, a 570 bp DNA fragment (part of the coat protein gene) was amplified from tomato samples showing yellow leaf curling. Its sequence was 96-98% homologous to that of a partially characterized geminiviruses described in tomatoes from Senegal (acc. nos. AF058029; D88800), and 83% homologous to TYLCV now present in many countries in the world (AF105975 and many others)

Study of the distribution of two biotypes of *Bemisia tabaci* (Gennadius) in Réunion Island using microsatellite markers

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Since 1997, Tomato yellow leaf curl virus (TYLCV) has been responsible of great losses on tomato production in Réunion Island. *Bemisia tabaci* infestations were not observed on vegetable crops before the detection of TYLCV in 1997, although *B. tabaci* had been reported several times in the island since 1938. After 1997, two genotypes of *B. tabaci* were differentiated in Réunion using RAPD-PCR and sequencing of a portion of a mitochondrial gene encoding cytochrome oxidase I. One was identified as biotype B and is supposed to have been recently introduced in Réunion, whereas the other is distinct from any genotype analysed so far. Since the latter genotype was also found in Madagascar, Mauritius and Seychelles, it was considered native from the south west of the Indian Ocean and was named Ms. The distribution of both biotypes was studied throughout Réunion using a genotyping technique based on microsatellites markers. *B. tabaci* was sampled in 30 different locations typical of the various ecosystems found in Réunion. Wherever possible the whiteflies were collected on crops and weeds to investigate an eventual host specificity of the biotypes.

Intra and interspecific competition between biotypes B and Q of *Bemisia tabaci*

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Bemisia tabaci individuals, biotypes B and Q, were established in single-biotype and mixed-biotype populations on 15-days-old tomato seedlings at 12, 24 and 48 whiteflies per seedling (50% females). After 6 days incubation (25°C, 60% RH and 16:8 hours (light:darkness)) the number of eggs and the leaf area were determined. F1 adults were collected daily and 30% of F1 individuals from mixed populations were analysed by RAPD-PCR to determine their biotype (n=587). The number of eggs per female and day and the number of F1 adults per female were higher for biotype B. Mortality of immature instars was higher for biotype Q. The higher fertility and fecundity would favour biotype B in sympatry with biotype Q. A linear relationship was established for biotype B between the number of eggs per female and day and the number of females per cm². Moreover, the number of F1 adults per female and the number of females per cm² were related only for biotype B in single population. Thus, moderate intraspecific competition for resources occurs for biotype B, which disappears when both biotypes are together, indicating interspecific competition favouring biotype B. Biotype B flies produced the highest percentage of F1 female while biotype Q produced more F1 females when reared in single colonies compared to mixed populations, suggesting interspecific competition by reproductive interference. Time to reach 50% F1 emergence was shorter for biotype B and the mixed biotypes than for biotype Q. Emergence time increased in proportion to the number of initial whiteflies, indicating intraspecific competition for both biotypes. The widespread presence of biotype Q in Spain suggests that competition is strongly modified in field conditions.

Population structure in *Bemisia tabaci* from Greece (Hemiptera: Aleyrodidae) based on microsatellite markers

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Microsatellites are short DNA fragments located throughout the genome which contain tandemly repeated patterns with two to six base-pairs. Because of their high polymorphism and co dominance these markers provide an effective tool of population genetic studies. We present here the identification and utilisation of polymorphic microsatellite loci in *Bemisia tabaci*. We used a biotin/streptavidin capture technique to isolate microsatellites directly from *B. tabaci* genomic DNA. Genomic libraries enriched for AC, AG, and AAC repeated motifs were made. Recombinant clones were screened using a three-primer PCR amplification test to detect microsatellites. After sequencing of the positive clones we obtained 37 microsatellite repeat regions. For 10 of them we designed species-specific flanking primers for microsatellite amplification; the remaining 27 are in progress of characterization. The polymorphism of the ten loci was examined initially in 6 populations from Crete. Eight over ten revealed a relatively high level of allelic diversity (7–14 alleles). Two loci revealed low polymorphism (2–3 alleles). Investigation of genotypic disequilibrium among the loci suggests that they were unlinked, carrying independent information across the samples. Genetic differentiation was analyzed by comparing genotypic distributions and computing F_{ST} estimates. Differentiation was significant ($F_{ST} = 0.21$, $P < 0.001$) over all samples. The analyses of the relationship between gene flow and geographic distance showed that there is no correlation between them. The cluster analysis based on Nei's genetic distances individualized a group containing all samples collected on *Ipomea indica* in different localities indicating that genetic exchanges among *B. tabaci* individuals from *Ipomea* are larger than with *B. tabaci* found on other plants. The microsatellite markers are currently being used to investigate the genetic variability of *B. tabaci* originated from different countries and/or belonging to different biotypes. They will be used in extensive studies for the analysis of the genetic structure and gene flow in *B. tabaci* populations in relation to several parameters: type of habitat (greenhouses versus open field) species of the host plant, geographic distance, population density, habitat discontinuities in space and time.
